South African Journal of Botany 88 (2013) 86–89

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Short communication

Larvicidal activity against *Anopheles arabiensis* of 10 South African plants that are traditionally used as mosquito repellents

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ARTICLE INFO

Article history:
Received 19 November 2012
Received in revised form 29 May 2013
Accepted 30 May 2013
Available online 28 June 2013

Edited by OM Grace

Keywords:
Anopheles arabiensis
Larvicides
Malaria
Mosquitoes
Olax dissitiflora

ABSTRACT

Ethanol extracts of *Aloe ferox*, *Atalaya alata*, *Balanites maughamii*, *Clausena anisata*, *Croton menyaarthii*, *Lippia javanica*, *Melia azedarach*, *Olax dissitiflora*, *Sclerocarya birrea* and *Trichilia emetica* were evaluated for their larvicidal activity against *Anopheles arabiensis* mosquitoes. Larval mortality was observed after 24 h of exposure. Larvicidal activity was only found in 5 plant extracts, namely, *C. anisata*, *C. menyaarthii*, *L. javanica*, *O. dissitiflora* and *T. emetica*. The bark extract of *O. dissitiflora* exhibited the highest larvicidal activity with LC_{50} value of 25.24 μg/ml. The results of the present study showed that the bark of *O. dissitiflora* may have the potential to be used as larvicides against *An. arabiensis*.

1. Introduction

Mosquito-borne diseases, such as malaria, Japanese encephalitis, filariasis, dengue and yellow fever remain a major source of illness and death worldwide, particularly in tropical and subtropical countries (Becker et al., 2003). Mosquitoes alone transmit diseases to more than 700 million people annually (Taubes, 2000). Among these diseases, malaria, which is caused by parasites of the genus *Plasmodium* and transmitted by infected mosquitoes of the genus *Anopheles*, continues to be a major public health problem in tropical and subtropical countries. In 2010, the World Health Organization (WHO) estimated that there were 216 million cases of malaria and 655 000 deaths worldwide. About 91% of these deaths occurred in sub-Saharan Africa, and were mostly in children under 5 years of age (WHO, 2011).

Since there is currently no effective vaccine available for the prevention of malaria, larviciding is one of the main strategies used to control the disease. Larviciding is a successful strategy of controlling mosquito borne diseases by killing mosquitoes at the larval stage before they emerge into adults. At present, mosquito larviciding depends primarily on the use of synthetic larvicides. Although effective, their repeated use has resulted in many problems, such as toxic effects to humans, non-target organisms, and the environment, and the development of resistance in mosquito populations (Das et al., 2007). These problems highlight the urgent need for development of new larvicides, which are effective, safe, biodegradable and target-specific.

Plants may be alternative sources of mosquito larvicidal agents because they constitute a rich source of bioactive chemicals (Sukumar et al., 1991). Natural products are generally preferred because they are less or not toxic to non-target organisms and are easily biodegradable (Rahuman et al., 2008). Much interest has, therefore, been focused on plant extracts or phytochemicals as potential sources of mosquito larvicidal agents or as lead compounds. A large number of plant extracts have been reported to possess larvicidal activity against mosquitoes (Ciccia et al., 2000; Rahuman et al., 2008; Kamaraj et al., 2010). The present study was undertaken to assess the larvicidal activity against *Anopheles arabiensis* of 10 extracts from South African plants that are reported to be used traditionally as mosquito repellents (Mavundza et al., 2011).

2. Materials and methods

2.1. Plant materials

An ethnobotanical survey of mosquito repellent plants was conducted in uMkhanyakude district, KwaZulu-Natal province, South Africa, between April and May 2011 (Mavundza et al., 2011). Fresh plant materials (Table 1) were collected from Ndumo village, in May 2012. Plant materials were identified by Dr. C.J. Potgieter of the Bews Herbarium, at the University of KwaZulu-Natal, Pietermaritzburg Campus, where voucher specimens are deposited.

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http://dx.doi.org/10.1016/j.sajb.2013.05.007

Table 1
Plants evaluated for larvicidal activity against *Anopheles arabiensis*.

Family	Botanical name	Common name	Local name	Voucher number	Part used
Xanthorrhoeaceae	*Aloe ferox* Mill.	Cape aloe	iNhlaba	EM08	Leaves
Anacardiaceae	*Sclerocarya birrea* (A. Rich.) Hochst.	Marula	Umango	EM10	Seeds
Balanitaceae	*Balanites maughamii* Sprague.	Torchwood	uGobendlovu	EM09	Bark
Euphorbiaceae	*Croton menyaarthii* Pax	Rough-leaved croton	Hubeshani	EM05	Leaves
Meliaceae	*Melia azedarach* L.	Chinaberry	Umsilinga	EM01	Leaves
Meliaceae	*Trichilia emetica* Vahl	Natal Mahogany	Umkhuhlu	EM06	Seeds
Olacaceae	*Olax dissitiflora* Oliver	Bastard sourplum	Mampuzane	EM04	Bark
Rutaceae	*Clausena anisata* (*Willd.*) *Hook.F.*	Perdepis	Umsanga	EM02	Leaves
Sapindaceae	*Atalaya alata* (*Sim*) *H.H.L. Forbes*	Lebombo krantz ash	Umnondo	EM07	Leaves
Verbenaceae	*Lippia javanica* (Brum.f) Spreng.	Fever tea	Umsuzwane	EM03	Leaves

2.2. Preparation of plant extracts

Plant materials were dried in an oven at 30–60 °C. The drying time and temperature varied depending on the nature of the plant material. The dried plant materials were powdered by an electrical blender. Plant samples (150 g) were extracted with 1 l of absolute ethanol. The extracts were filtered through Whatman No. 1 filter paper and concentrated under vacuum using a rotary evaporator (Büchi, Germany) at 30 °C. The concentrated extracts were dried at room temperature and then stored at 4 °C for later use.

2.3. Mosquitoes

The larvicidal activity of plant extracts was evaluated against laboratory-reared larvae of *An. arabiensis* mosquitoes, a potent malaria vector in South Africa. *An. arabiensis* larvae were obtained from a permanent colony maintained at 27 ± 2 °C and 85% relative humidity in the insectary of the Malaria Research Unit, Medical Research Council, Durban, South Africa. Larvae were fed on dog biscuits and yeast powder at a 3:1 ratio. Adults were provided with a 10% sucrose solution. Female mosquitoes were periodically blood-fed on restrained albino guinea pigs for egg production. The guinea pigs were reared according to the National Research Council's guidelines for the care and use of laboratory animals (National Research Council, 1996).

2.4. Larvicidal assay

The larvicidal activity of plant extracts was evaluated according to the protocol of the World Health Organization (WHO) with slight modifications (WHO, 2005). Briefly, each extract was dissolved in acetone to prepare a stock solution of 50 mg/ml. One ml of each stock solution was added to 99 ml of distilled water in a 500 ml plastic beaker to obtain a test concentration of 500 μg/ml. Twenty five third instar larvae of *An. arabiensis* were then introduced in that beaker. The larvae were fed dry yeast powder. Acetone and distilled water were used as negative controls, while Temephos (Abate®) was used as a positive control. The number of dead larvae was recorded after 24 h of exposure and the percentage mortality was calculated. The larvae were considered dead if they did not move when prodded with a needle in the siphon or cervical region. Each extract was tested in triplicate and the assay was repeated twice. The extracts showing 100% mortality were selected for a dose–response bioassay.

2.5. Dose–response bioassay

Based on the preliminary screening results, crude extracts of *Clausena anisata* and *Olax dissitiflora* were subjected to a dose–response bioassay to determine the concentration required to kill 50% (LC_{50}) of the *An. arabiensis* larvae. The extracts were tested at different concentrations ranging from 15.6 to 500 μg/ml. The number of dead larvae was recorded after 24 h of exposure and the percentage mortality was calculated. Each concentration was tested in triplicate and the assay was repeated twice.

2.6. Data analysis

LC_{50} values (lethal concentration that caused 50% larval mortality) were determined by Probit analysis using SPSS version 12 software. Results with $p < 0.05$ were considered to be statistically significant.

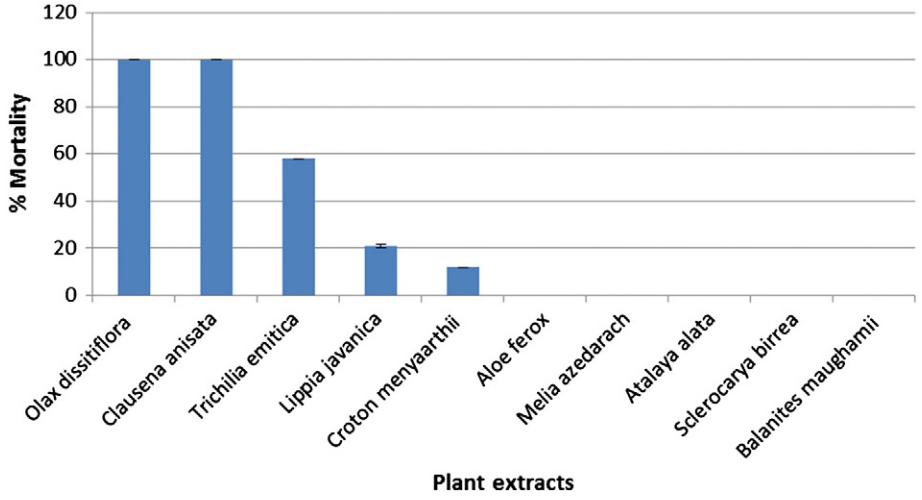

Fig. 1. Larvicidal activity of ethanolic plant extracts against the third instar larvae of *An. arabiensis* at 500 μg/ml.

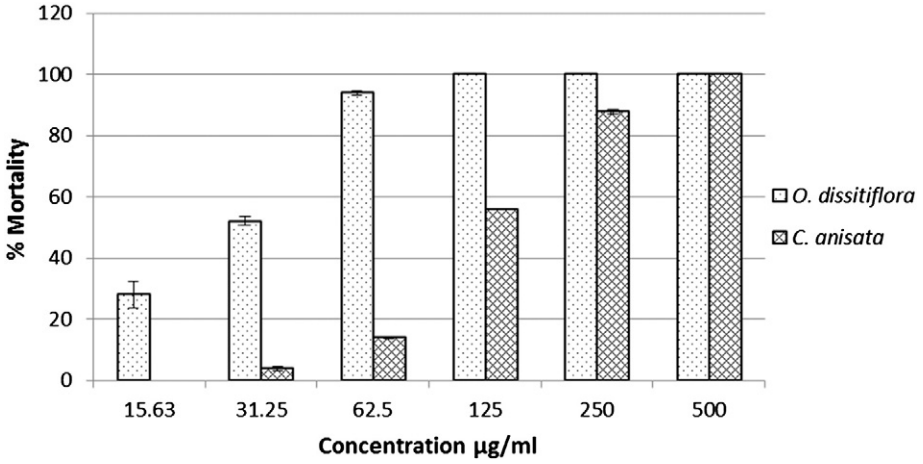

Fig. 2. Larvicidal activity of ethanolic bark extract of *O. dissitiflora* and leaves of *C. anisata* against the third instar larvae of *An. arabiensis* at concentrations ranging from 15.6 to 500 µg/ml.

3. Results and discussion

The larvicidal activity of extracts varied according to plant species (Fig. 1). Among the extracts tested in the present study, larvicidal activity was found in only five extracts. No larval mortality was observed in the negative control, while the positive control exhibited 100% larval mortality. Of the active extracts, the bark extract of *O. dissitiflora* and leaf extract of *C. anisata* exhibited the highest larvicidal activity (100%), followed by seed extract of *Trichilia emetica* (58%), leaf extract of *Lippia javanica* (21%) and *Croton menyaarthii* (12%). Our findings are similar to those of Karunamoorthi and Ilango (2010) who reported the larvicidal activity of methanol leaf extract of *Croton macrostachyus* against *An. arabiensis*. Other plant taxa which have been found to larvicidal activity against *An. arabiensis* include ethanol extracts of the leaves of *Ricinus communis* (Euphorbiaceae), *Catharanthus rosea* (Apocynaceae) and *Lantana camara* (Verbenaceae) (Taha et al., 2011). Larvicidal activity of a water extract of the leaves of *R. communis* against *An. arabiensis* has also been reported (Elimama et al., 2009). The activity of crude plant extracts is often attributed to the complex mixture of active compounds (Kamaraj et al., 2011). Various compounds such as phenolics, terpenoids, and alkaloids, which are found in plants, jointly or individually contribute to the larvicidal activities against mosquitoes (Jang et al., 2002).

At 500 µg/ml, the leaf extracts of *Aloe ferox*, *Melia azedarach*, *Atalaya alata*, the seed extract of *Sclerocarya birrea* and bark extract of *Balanites maughamii* did not show any larvicidal activity. According to Jang et al. (2002), most plants with mosquito larvicidal activities belong to the families Apiaceae, Araceae, Magnoliaceae, Piperaceae, Rutaceae, and Zingiberaceae. In contrast to our findings, larvicidal activity of extracts of *M. azedarach* against different species of mosquitoes has been reported (Wandscheer et al., 2004; Nathan et al., 2006; Coria et al., 2008). Maharaj et al. (2012) reported the larvicidal activity of *A. ferox* against *An. arabiensis* mosquitoes. We attribute the absence of larvicidal activities of the plant extracts to natural variation in plant species, the plant part used, its geographical location, as well as methodological differences in the application and mosquito species used (Sukumar et al., 1991; Das et al., 2007).

Two species included in this study gave convincing 100% larval mortality effects in the dose–response bioassay. The bark extract of *O. dissitiflora* exhibited 100% larval mortality to a lower concentration of 125 µg/ml and 94% at 62.5 µg/ml. The leaf extract of *C. anisata* yielded larval mortality of 100% at 500 µg/ml and 88% at 250 µg/ml (Fig. 2). Of these two extracts, the bark extract of *O. dissitiflora* exhibited the higher larvicidal activity with LC_{50} value of 25.2 µg/ml compared to the leaf extract of *C. anisata* which had an LC_{50} value of 112.7 µg/ml. Govindarajan (2010) reported that the essential oil from the leaves of *C. anisata* exhibited LC_{50} values of 140.96, 130.19 and 119.59 ppm against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephenis*, respectively. *C. anisata* has been reported to contain many carbazole alkaloids and coumarins (Ngadjui et al., 1989; Songue et al., 2012). It is possible that the larvicidal activity shown by leaf extract of *C. anisata* may be attributed to these compounds. To the best of our knowledge, the larvicidal activities of *O. dissitiflora* have never been reported before. There is also no report on the chemical constituents of *O. dissitiflora*.

4. Conclusions

Our results showed that the ethanolic extracts from the bark of *O. dissitiflora* and leaves of *C. anisata* have the potential to be used as larvicides against *An. arabiensis*. Further studies to isolate and identify the active compounds are in progress. Studies to evaluate the larvicidal activity of the bark extracts of *O. dissitiflora* against other mosquito species are also being considered. The results of the present study could be useful in promoting research aimed at the development of new agents for mosquito control based on bioactive chemical compounds from indigenous plant sources.

Acknowledgments

The authors are grateful to Dr. Christina Potgieter of the Bews Herbarium, School of Life Sciences, University of KwaZulu-Natal, for her valuable assistance with plant identification. Thanks are also due to Mr. Jabulani Zikhali and Ms. Reshma Gayaram for their assistance with the collection of plants and larvicidal assay, respectively. Last but not least, the authors are grateful to the South African Medical Research Council for financial support.

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